"~~Anti-retroviral functionalized aromatic compounds~~".

The subject of the invention is the use of functionalized aromatic compounds for inhibiting the retroviral Tat protein.

The present invention also relates to novel triphene derivatives, the method for preparing them as well as their use as medicament in particular for the treatment of viral infections such as acquired immunodeficiency syndrome (AIDS).

The human immunodeficiency virus (HIV) is a retrovirus of the group comprising lentiviruses known for their variability within the same host cell during the progression of the infection, and for their constant replication throughout the disease.

The HIV viral cycle starts with the attachment of the virus to a host cell. The virus then penetrates into the cytoplasm of the cell where it is decapsidated. The RNA released is transcribed into double-stranded DNA by means of reverse transcriptase. Once the proviral DNA has been integrated into the cellular genome, the viral replication is activated by a viral protein, the Tat (Trans-Acting Transcriptional activator) protein. The viral cycle ends with the budding of new viral particles at the surface of the cell.

The only therapeutic agents which have proved effective against AIDS are agents which block the human immunodeficiency virus (HIV) viral cycle. Two types of anti-retroviral agents are currently marketed:

- the nucleoside analog inhibitors of reverse transcriptase, such as zidovidine (AZT), didanosine, stavudine, zalcitabine, and
- the antiproteases, which are inhibitors of the viral protease responsible for the formation of virions; these antiproteases, such as saquinavir, ritonavir and indinavir, act at a later phase of the viral cycle than does AZT.

The enzymes for viral replication, such as reverse transcriptase, RNA polymerase and protease, have the characteristic feature of committing a number of major errors, with a rapid rate of replication, such that HIV possesses high mutation and recombination rates.

Accordingly, the efficiency of the therapeutic agents used to block the viral cycle decreases with the cycles, following the appearance of resistant strains among the variants.

To combat the appearance of resistant strains, the use of several anti-retroviral agents, each acting at a different stage of the viral cycle, is for the moment the most effective solution.

Some combinations have made it possible to obtain remarkable results with a practically undetectable viremia in some patients. However, stopping the treatment shows that the viremia rises again in a spectacular manner. Reservoir cells and the absence of a reaction of certain lymphocytic lines (CTL) responsible for eliminating cells contaminated with HIV therefore remain major problems.

Furthermore, numerous patients have followed monotherapies and have therefore developed resistance. In these patients, multitherapies have much more limited effects.

The present invention relates to novel types of agent blocking the viral cycle, both by their chemical structure and by their mode of action. These novel anti-retroviral agents are specific inhibitors of the viral Tat protein.

The Tat protein has already been the subject of numerous studies. The *tat* gene is composed of two exons encoding a protein of 86 to 102 residues according to the isolates.

The Tat protein is essential for the expression of the HIV-1 viral genome (Arya, S.K., Guo, C., Josephs, S.F., & Wong-Staal, F. (1985) Science 229, 69-73).

It is known that the viral replication is triggered by Tat according to a transactivation mechanism. The beginning of the viral mRNA carries a sequence termed TAR (RNA Trans Activation Response Element) in the form of a loop onto which the TRBP protein (TAR Binding Protein) attaches and blocks the action of RNA polymerase by complexing it. The basic region of Tat adopts an extended structure, attaches to TAR, displaces TRBP and releases the RNA polymerase, such that transcription can begin (Berkhout, B., A. Gatignol, A.B. Rabson, and K.-T Jeang, (1990) Cell 62, 7257-7267; Loret, E.P., Georgel, P., Johnson, W.C., & Ho, P.S. (1992) Proc. Natl. Acad Sci. USA 89, 9734-9738).

It has now been demonstrated that the Tat protein is involved in the deregulation of numerous cellular functions and in some infection-related biologies. Tat is prematurely expressed by the virus genome.

Tat is found both inside cells and in the extracellular medium. Its action is not restricted to the cells in which it is produced because it can cross the cytoplasmic membrane and penetrate into other infected or noninfected cells. Nanomolar concentrations of Tat are detected in the serum of patients infected with HIV-1 (Westendorp, M.O., Frank, R., Ochsenbauer, C., Stricker, K., Dhein, J., Walczak, H., Debatin, K.M. & Krammer, P.H. (1995) Nature 375, 497-500).

In synergy with bFGF, Tat promotes the development of Kaposi's syndrome observed in numerous AIDS patients (Ensoli, B., Genselman, R., Markham, P., Fiorelli, V., Colombini, S., Raffeld, M., Cafaro, A., Chang, H.K., Brady, J.N., & Gallo, R.C. (1994) Nature 371, 674-680).

Tat also exhibits a specific cytotoxic activity on certain lymphocytic lines (Westendorp, M. O., Shatrov, V.A., Schulze-Osthoff, K. Frank, R., Kraft, M., Los, M., Krammer, P.H., Dröge, W., & Lehmann, V. (1995) EMBO 14, 546-554) and possesses an

immunosuppressive activity by repressing the MHC (Howcroft, T.K., Strbel, K., Martin, M.A., & Singer, D.S. (1993) Science 260, 1320-1322).

This property of crossing the membranes is partly due to its basic region (Vivès, E., Brodin, P., & Lebleu, B. (1997) J Biol Chem. 272, 16010-16017; Efthymidias, A., Briggs, L. J., & Jans, D.A. J Biol Chem. 273, 1623-1628 (1998)).

The presence, at the N- and C-terminal ends of Tat, of sequences preventing or slowing down its digestion by exoproteases makes it possible to explain that Tat is not degraded in extracellular medium (Loret, E.P., Vives, E., Ho, P.S., Rochat, H., Van Rietschoten, J., & Johnson, W.C. (1991) Biochemistry 30, 6013-6023).

Recently, the role of Tat was also demonstrated in the reverse transcription of viral RNA (Harrich, D., Ulich, C., Garcia-Martinez, L.F., & Gaynor, R.B. (1997) EMBO 16, 1224-35).

Generic antagonists of the action of Tat (antisense RNA, RNA traps, transdominant mutants) which block transactivation by Tat in vitro have proved ineffective in vivo (Pearson, L., Garcia, J., Wu, F., Modesti, N., Nelson, J., * Gaynor, R. (1990) Proc. Natl. Acad. Sci. USA 87, 5079-5083; Chang, H.K., Gendelman, R., Lisziewicz, G., Gallo, R.C., * Ensoli, B., (1994) Gene Ther 1, 208-216).

M.C. HSU et al. in "Science,254 (1991), 1799-1802" and then in "Proc. Natl. Acad. Sci. USA, 90 (1993), 6395-6399" have shown the inhibitory effect of benzodiazepine on the activity of Tat. However, these derivatives act on a cellular factor involved in the Tat function and not on Tat itself.

Other transactivation inhibitors were described more recently, such as the derivatives of Quinacrine and Chloroquine (Jiang, M.C., Lin, J.K., Chen, S.L. (1996) Biochem Biophys. Res. Commun.226, 1-7) or alternatively derivatives of Fluoroquinoline, in particular a molecule called K-12 (Baba et al. (1998)

Mol. Pharamcol.6, 1097-1103). However, inhibitors are not specific for Tat, and K-12 has a very broad antiviral activity ranging from HIV-1, HIV-2, SIV to the herpesvirus or the varicella virus (Witrouw et al. (1998) Antivir, Chem. Chemother., 5, 403-411) which shows that K-12 probably acts on a cellular factor.

Finally, LAPIDO et al. (FEBS Letters, 367 (1995), 33-38) have described a tetrahydropyridine derivative capable of attaching to a polyarginine peptide comprising nine basic arginine residues. This peptide is capable of binding to TAR but it appears difficult to extropolate these results to the whole Tat protein which does not comprise a sequence having nine arginines.

No molecules therefore exist in the prior art which are capable of attaching to the Tat protein and of inhibiting its activity in vitro.

The Tat protein has been the subject of several structural studies.

The basic region of Tat, in the form of an extended structure, inserts into the major groove of TAR without modifying the type A helix which the polynucleotide forms (Loret, E.P., Georgel, P., Johnson, W.C., & Ho, P.S. (1992) Proc. Natl. Acad Sci. USA 89, 9734-9738). A preliminary study by 2D NMR of the African variant Tat Z2 has shown that the basic region was closed to the N-terminal region. However, the small number of NMR constraints has not made it possible to determine a precise 3D structure of Tat (Bayer, P., Kraft, M., Ejchart, A., Westendorp, M., Frank, R., & Rosh, P. (1995), J. Mol. Biol. 247, 529-535).

A structural study by circular dichroism and molecular modeling have made it possible to demonstrate the existence of 6 structural groups among the known variants of the Tat protein, and the structural variations are mainly located in two regions adjacent to the basic region (Gregoire & Loret, J. Biol. Chem., 271 (1996), 22 641-22 646).

One of the main conclusions of this study have been to postulate that conformational changes were essential for the Tat protein for its transactivation activity. Indeed, the basic region of Tat cannot again find itself inserted into the major groove of TAR in its conformation in solution. The regions adjacent to the basic region probably play a role of "hinge" regions to allow this insertion into TAR.

Attempts to find an inhibitor of the transactivation of HIV have up until now come up against the high affinity of the Tat/TAR interaction, which is nanomolar. Since the end of the 80s, several teams have succeeded in synthesizing molecules which attach to TAR, but these molecules, even equipped with a nanomolar affinity, do not succeed in being true competitive inhibitors of Tat.

Jms A5 The object of the present invention is to provide allosteric inhibitors of the Tat protein. These molecules attach to different regions of Tat. This attachment prevents the conformational changes essential to the Tat protein for its transactivation activity.

A first advantage of this approach is to avoid the problems of competitive inhibitor with TAR which are similar to those of the competitive inhibition with Tat. A second advantage is to make it possible to inhibit the other functions of Tat both at the intracellular and extracellular level.

Jms A6 The present invention relates to the use of an organic compound comprising an aromatic ring, noted Ar, substituted with at least one hydrocarbon substituent noted A, said hydrocarbon substituent comprising:

- a nonfunctionalized linear aliphatic chain noted $-CH_2A'$ comprising at least one carbon atom, and
- a substituent noted F_a comprising at least one proton donor or acceptor function capable of establishing one or more hydrogen bonds,

- in order to bring about the allosterinc inhibition of the Tat protein.

The compound according to the invention preferably links the basic region and the N-terminal region of the Tat protein, such that the structure of the protein is rigidified, and its conformational change, necessary to interact with TAR, is inhibited.

In the context of the present invention, "Tat protein" is understood to mean a succession of amino acids bringing about the tranactivation of the HIV genes and capable of comprising mutations.

In the context of the present invention, the complete solid-phase chemical synthesis of six structural variants of Tat has been carried out using HIV-1 isolates of different geographical origins (Africa, Europe, North America): Tat Z2 (86 residues), Tat Mal (87 residues), Tat Bru (86 residues), Tat JR (101 residues), Tat Oyi (101 residues) and Tat Eli (99 residues). These molecules have properties which are identical to the "natural" Tat proteins. Their functional study makes it possible to demonstrate a close link between the virulence if HIV and the activity of the Tat protein.

In the context of the present invention, the "Tat protein" groups together in particular the Tat Z2, Tat Mal, Tat Bru, Tat JR, Tat Oyi and TatEli variants.

"Hydrocarbon" is understood to mean a group of atoms comprising a carbon atom directly attached to the rest of the molecule, and optionally attached to the rest of the molecule, and optionally one or more heteroatoms inserted into the carbon backbone.

In order to take into account the structural heterogeneity of Tat, the hydrocarbon susbstituent A is sufficiently flexible to adapt to the slight structural modifications of Tat from one variant to another.

The aromatic ring Ar is a derivative of toluene or a condensed polycyclic aromatic hydrocarbon, preferably chosen from naphthalene, anthracene, phenanthrene, fluoranthene, aceanthrylene and triphene.

The aromatic ring is preferably triphene.

In the context of the present invention, a study by heteronuclear (H and ^{13}C) 2D NMR was carried out on Tat Bru. It has been possible to observe a 3D structure of Tat Bru preserving 950 NMR constraints. This structure makes it possible in particular to visualize the position of the side chains and of the substantial modifications in the coiling of the peptide backbone relative to the 2D NMR study previously carried out on Tat Z2. An advantageous feature of the 3D structure of Tat Bru is the demonstration of an accessible hydrophobic "pocket" formed by tryptophan No. 11 (Trp 11) and phenylalanine No. 38 (Phe 38). These two residues are conserved in all the Tat variants.

According to a preferred embodiment, the aromatic ring of the compound of the invention interacts with tryptophan No. 11 (Trp 11) and phenylalanine No. 38 (Phe 38) of Tat.

Advantageously, the substituent F_a establishes one or more hydrogen bonds with a basic region and the N-terminal region of the Tat protein.

The proton donor function an acceptor function is chosen from all the proton donor and acceptor functions well known to persons skilled in the art. The alcohol function is for example chosen as the proton donor function, in particular a primary alcohol or secondary alcohol function, and the carbonyl function as proton acceptor function.

The proton donor or acceptor function of the substituent F_a is advantageously situated at a distance of between 5 and 10 Å of the aromatic ring, preferably of between 6 and 7 Å.

The aromatic ring is advantageously such that the nonfunctionalized linear aliphatic chain noted $-CH_2A'$ comprises1 to 8 atoms, among which carbon atoms and optionally one or two heteroatoms. Heteroatom is understood to mean an atom other than carbon, for example N, P, O, S, Si or Se.

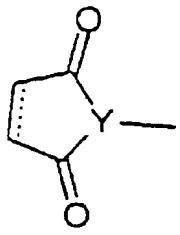
According to a first embodiment, $-CH_2A'$ comprises a carbon atom and F_a represents a hydroxyl, such that A represents $-CH_2OH$.

The nonfunctionalized linear aliphatic chain $-CH_2A'$ advantageously comprises 5 carbon atoms.

According to a second embodiment, the substituent F_a situated at the end of the aliphatic chain comprises at least one proton acceptor function, preferably at least two, which are situated in the plane of the aromatic ring and on the same side of the plane of the aromatic ring.

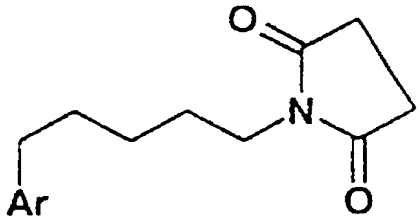
A preferred proton acceptor function is a carbonyl function.

The substituent F_a situated at the end of the aliphatic chain advantageously corresponds to the formula:



in which Y represents N or CH and the dotted line represents a possible double bond. In this case, F_a is preferably a maleimide or a succinimide.

A preferred compound is such that the substituent F_a represents a maleimide or a succinimide and $-CH_2A'$ comprises 5 carbon atoms. This compound has the following formula:



In this formula, the aromatic ring Ar is preferably triphene.

The compound of the invention which comprises an aromatic ring Ar substituted with A may comprise in addition at least one other substituent noted B or C,

it being possible for the said substituent to comprise at least one carbon atom, and to comprise a substituent noted F_b or F_c comprising at least one proton donor or acceptor function capable of establishing one or more hydrogen bon with the Tat protein.

The aromatic ring Ar advantageously comprises, in addition to the substituent A, two substituents B and C.

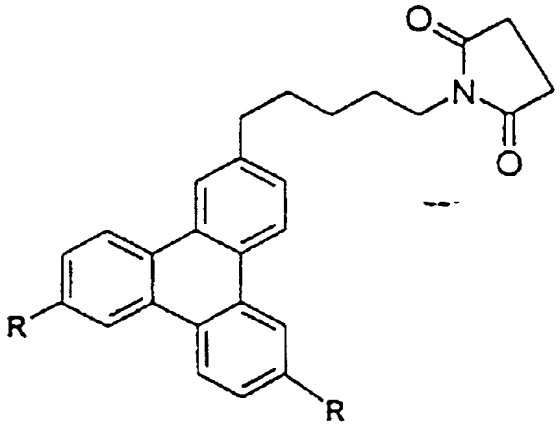
B or C may comprise at least one donor or acceptor function, such as a hydroxyl function.

B or C advantageously represents a methyl, $-CH_2OH$ or -COOH.

B or C may comprise two proton donor acceptor functions situated

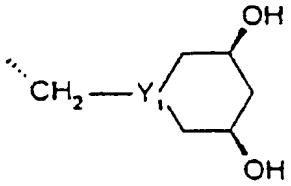
- in the plane of the aromatic ring, or
- on the same side of the plane of the aromatic ring, for the functions to effectively interact with Tat.

The organic compound advantageously corresponds to the formula:



in which R represents a hydrogen, a methyl (compound noted TDS1), $-CH_2OH$ (compound noted TDS4), or

the group of formula



in which Y1 represents N (compound noted TDS2), or CH (compound noted TDS3).

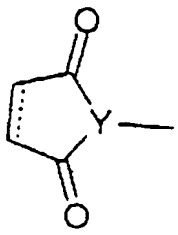
The present invention also relates to the novel derivatives of triphene which are substituted with a hydrocarbon substituent A comprising a nonfunctionalized linear aliphatic chain and, at the end of the chain, a substituent comprising at least one function provided with a proton acceptor or donor doublet, with the exception of the triphene derivatives substituted at the 2-position with $-CH_2-NH-CH2-CH(OCH_3)_2$ or $-CH_2-NH-C(CH_3)(CH_2OH)_2$.

The nonfunctionalized linear chain of these triphene derivatives preferably comprise up to 8 atoms.

The substituent situated at the end of the linear aliphatic chain may comprise at least two functions provided with a proton acceptor doublet, preferably situated in the same plane.

The function provided with a proton acceptor doublet is advantageously a carbonyl.

The substituent situated at the end of the aliphatic chain advantageously corresponds to the formula:



in which Y represents N or CH and the dotted line represents a possible double bond. This substituent preferably represents a maleimide or a succinimide.

The invention also relates to novel di- or trisubstituted triphene derivatives comprising a hydrocarbon substituent A as described above, and comprising at least a second substituent B or C.

The linear aliphatic chain of A comprises up to 8 atoms, among which carbon atoms and optionally one or more heteroatoms, preferably 5 atoms.

The substituent situated at the end of the linear aliphatic chain comprises at least two functions

provided with a double proton acceptor, preferably in the same plane.

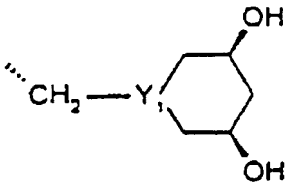
The functions provided with a double proton acceptor is advantageously a carbonyl.

B and/or C are, independently of each other, aliphatic substituents comprising 1 to 4 carbon atoms, for example a methyl, and may be provided, independently of each other, with at least one proton donor or acceptor function.

B and/or C are, independently of each other, provided with two proton donor or acceptor functions preferably positioned in space such that the functions are situated

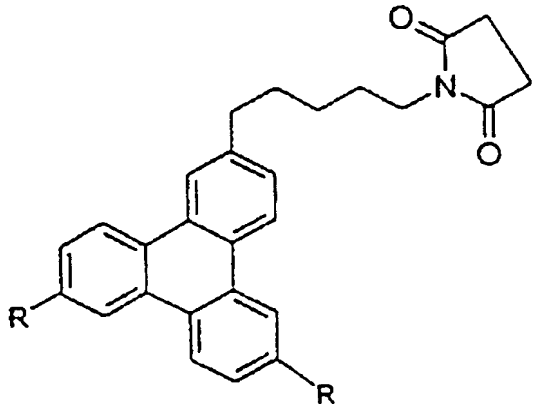
- in the plane of the trephine nuclear, or
- on the same side of the plane of the triphene nuclear.

The present invention relates particularly to trisubstituted triphene derivatives such that B and C represent a methyl, a hydroxymethyl or the following group:



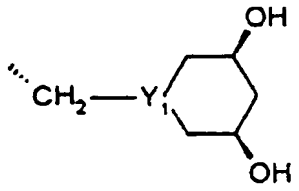
Y1 being a nitrogen atom or a CH group.

The present invention covers more particularly the derivatives of formula



in which R represents a methyl (compound noted TDS1), $-CH_2OH$ (compound noted TDS4), or

the group of formula



in which Y1 represents N (compound noted TDS2), or CH (compound noted TDS3).

The present invention also relates to the 2,6,10-trihydroxymethyltriphene derivative and the 2,6,10-tricarboxytriophene derivative. These derivatives are useful as synthesis intermediates in the production of the compounds described above.

In the compounds of the invention, the substituents A, B and C are preferably in the ortho or meta position respectively. The compounds of the 2-A-6-B-10-C-triphene type will be preferred so as to minimize the possible interactions between A, B and C.

The subject of the present invention is also a method of preparing the compounds described above, in particular the aromatic compounds trisubstituted with the A, B and C groups noted Ar(ABC), and the aromatic compounds monosubstituted with A noted ArA.

The present invention relates more particularly to the method of preparing the aromatic compounds Ar(ABC) and ArA, for which A (noted CH_2-A'F_a) comprises a nonfunctionalized linear aliphatic chain (CH_2-A'), substituted at its end with a group provided with at least one proton acceptor or donor function (F_a).

The method of preparing the compounds Ar(ABC) preferably uses, as intermediate products, a derivative of formula P_aA'-H_2C-Ar-$(CH_2Z)_2$ in which -CH_2A' is as defined above, P_a represents a hydrolyzable protective group and Z represents a hydrogen, halogen or a protected alcohol function.

Z is preferably a bromine or a trialkylsilyloxy group.

The method of the invention preferably uses, as starting material, Ar$(CH_3)_3$.

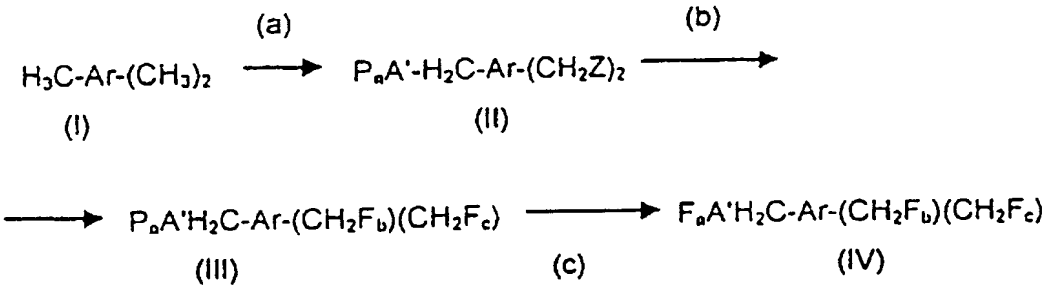
In order to preserve the reactivities of the substituents A, B and C, and to avoid their degradation during the synthesis, the method according to the invention preferably comprises the following successive steps:

(a) attachment of the nonfunctionalized linear aliphatic chain $-CH_2A'$,

(b) possible attachment of the substituents B and C, and

(c) attachment of a substituent comprising at least one proton acceptor or donor function F_a to the nonfunctionalized chain $-CH_2A'$.

According to a first embodiment for preparing the compounds Ar(ABC) such that B and C do not represent hydrogen, the method may be schematically represented as follows



F_a, F_b, F_c representing substituents comprising at least one proton acceptor or donor function

The derivative $P_aA'-H_2C-Ar-(CH_2Z)_2$ (II) is advantageously obtained by magnesian synthesis, using the compound of formula $P_aA'-MgX^1$, in which X^1 is a halogen atom. P_aA-MgX^1 is for example $BnO-(CH_2)_n-MgBr$, n being greater than 1, and Bn representing a benzyl.

The compound Ar(ABC) such that B and C represent Me are obtained from $Ar(Me)_3$ by carrying out steps (a) and (c). In this case, Z=H. Step (a) consists in breaking the symmetry of the starting material by carrying out a monohalogenation of $Ar(Me)_3$ in order to obtain $(X^2-H_2C)-Ar-(Me)_2$, X^2 representing a halogen. The monohalogenation is carried out for example with N-bromosuccinimide by catalysis with AIBN.

The chain A' is then grafted onto $(X^2\text{-}H_2C)\text{-}Ar\text{-}(Me)_2$ by magnesian synthesis as described above in order to obtain $P_aA'\text{-}H_2C\text{-}Ar\text{-}(Me)_2$. Step (c) consists in converting $P_aA'\text{-}H_2C\text{-}Ar\text{-}(Me)_2$ into $F_aA'\text{-}H_2C\text{-}Ar\text{-}(Me)_2$, F_a representing the group provided with at least one proton acceptor or acceptor function.

If it is desired to prepare a compound Ar(ABC) such that the groups B and C each comprise at least one proton acceptor or donor function (F_b and F_c), [...?. There is another voice on the whole of this tape which is getting lounder all the time!] will be distinguished depending on whether the bonds established by F_b and F_c with $A\text{-}Ar\text{-}(CH2\text{-})_2$ are carbon-carbon, carbon-nitrogen or carbon-oxygen bonds.

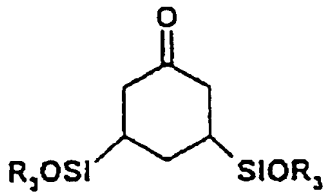
When the bonds between F_b, F_c and $A\text{-}Ar\text{-}(CH2\text{-})_2$ are carbon-carbon bonds, the intermediate $P_aA'\text{-}H_2C\text{-}Ar\text{-}(CH_2Z)_2$ (II) is such that Z represents a protected alcohol function or a halogen.

During steps (b), F_b and F_c may be grafted according to a Wittig reaction in two ways:

- an ylide derived from $P_aA'\text{-}H_2C\text{-}Ar\text{-}(CH_2Z)_2$ (II) when Z represents a halogen is reacted with a ketone comprising at least one proton donor or acceptor function F_b and/or F_c, or
- an aldehyde obtained by oxidation of $P_aA'\text{-}H_2C\text{-}Ar\text{-}(CH_2Z)_2$, when Z represents a protected alcohol function, is exposed to ylide precursors of F_b and F_c.

The ylide is obtained from $P_aA'\text{-}H_2C\text{-}Ar\text{-}(CH_2Z)_2$ when Z represents a halogen, directly or via $P_aA'\text{-}H_2C\text{-}Ar\text{-}(CH_2SO_2Ph)_2$ (Julia reaction).

For example, the compound $P_aA'\text{-}H_2C\text{-}Ar\text{-}(CH_2Br)_2$ is reacted with PPh_3 in DMF, and then nBuLi is added in order to obtain $P_aA'\text{-}H_2C\text{-}Ar\text{-}(Ph_3P{=}CH_2)_2$. There is then added the ketone in THF, for example of formula



in which R represents an alkyl substituent.

This ketone may be obtained from 1,3,5-cyclohexanetriol, which is treated with 2 equivalents of $tBuMe_2SiCl$ in CH_2Cl_2 in the presence of imidazole, and then by oxidation with the Dess-Martin reagent.

When the bonds between F_b, F_c and A-Ar-$(CH-)_2$ are carbon-nitrogen bonds, the intermediate $P_aA'-H_2C-Ar-(CH_2Z)_2$ (II) is such that Z represents a halogen or a protected alcohol function. Two routes are possible for the synthesis of this intermediate.

According to a first embodiment, Z represents a halogen and the method comprises the following steps:

$$Ar(Me)_3 \rightarrow X^3H_2C-Ar-(Me)_2 \rightarrow P_aA'-H_2C-Ar-(Me)_2 \rightarrow P_aA'-H_2C-Ar-(CH_2Z)_2$$

X^3 representing a halogen.

According to a second embodiment, Z represents a protected alcohol function, the method comprises the following steps:

$$Ar(Me)_3 \rightarrow HOH_2C-Ar-(CH_2OH)_2 \rightarrow HOH_2C-Ar-(CH_2Z)_2$$
$$\rightarrow X^4H_2C-Ar-(CH_2Z)_2 \rightarrow P_aA'-H_2C-Ar-(CH_2Z)_2$$

X^4 representing a halogen.

According to the second embodiment, the alcohol functions may be deprotected and then subjected to a sourse of halide in order to obtain $P_aA'-H_2C-Ar-(CH_2Z)_2$ in which Z represents halogen.

$HOH_2C-Ar-(CH_2OH)_2$ may be prepared in two different ways. 2,6,10-Trihydroxymethyltriphene may be obtained according to two routes. According to a first variant, 2,6,10-trimethyltriphene is tribrominated, for example with N-bromosuccinimide, and then hydrolyzed in a basic medium. According to a second variant, 2,6,10-trimethyltriphene is exposed to oxidants, for example $Na_2Cr_2O_7$, and the derivative

2,6,10-tricarboxytriphene obtained is reduced, for example with $AlLiH_4$.

The compound $P_aA'-H_2C-Ar-(CH_2Z)_2$ such that Z represents a halogen is then reacted, in a conventional manner, with a compound of the secondary amine type comprising at least one proton donor or acceptor function F_b and/or F_c; this reaction which follows a nucleophilic substitution mechanism is advantageously carried out in the presence of a base, such as TEA. For example, 2,4-dihydroxycyclohexamine is chosen as amine.

When the bonds established F_b and F_c with $A-Ar-(CH_2-)_2$ are carbon-oxygen bonds, the intermediate $P_aA'-H_2C-Ar-(CH_2Z)_2$ is such that Z represents a protected alcohol function. The intermediate is then obtained according to the scheme presented above.

In the case where B and C represent CH_2OH, F_b and F_c represent OH and step (b) consists in deprotecting the alcohol function of $P_aA'-H_2C-Ar-(CH_2Z)_2$.

When F_a represents a maleimide or a succinimide, step (c) follows the conditions of the Mitsunobu reaction.

For example, $BnO-(CH_2)_n-Ar-(Me)_2$, n being as defined above, is deprotected by hydrogenolysis of the benzyl group, in particular by dissolving it in a methanol/toluene mixture and by reacting it for 24 hours under a H_2 atmosphere in the presence of 10% Pd/C. $HO-(CH_2)_n-Ar-(Me)_2$ is then reacted, for example, with N-bromosuccinimide or N-bromomaleimide, in the presence of triphenylphosphine (PPh_3) and DEAD, in THF at room temperature for 12 hours.

The present invention relates in particular to a method of preparing aromatic compounds comprising a triphene nucleus.

According to an embodiment which is simple to carry out, the method uses trimethyltriphene, preferably 2,6,10-trimethyltriphene, as starting material.

The present invention also relates to a method of preparing the compounds ArA such that A represents a nonfunctionalized linear aliphatic chain substituted at its end with a group provided with at least one proton acceptor or acceptor function.

These compounds may be obtained by a Friedel-Crafts reaction. The method of the invention advantageously uses Ar-A'CH_2OH as precursor.

According to another method, ArH is monohalogenated and then converted to an intermediate of the ArA'CH_2CN type.

The present invention also relates to the compounds described above which are capable of being obtained by the method described above for their application as therapeutically active substances, in particular as anti-retroviral agents for the treatment or the prevention of infections due to a retrovirus, for example HIV.

The subject of the present invention is the pharmaceutical preparations containing a compound of the invention capable of being obtained by the method described above and containing a pharmaceutically inert excipient.

The subject of the present invention is finally the pharmaceutical preparations containing a mixture of a compound of the invention and of another anti-retroviral agent, as combination product for use simultaneously, separately or spaced out over time in an anti-retroviral therapy.

The present invention will be illustrated with no limitation being implied by the following examples with reference to Figures 1 to 7.

Figure 1 represents the HPLC profiles of TDS1, of Tat Bru, Tat Oyi and TDS1/Tat Bru and TDS1/Tat Oyi mixtures.

Figure 1A represents the HPLC profile of TDS1 at a concentration of 0.1 mM.

Figure 1B represents the HPLC profile of the Tat Bru protein at a concentration of 0.1 mM.

Figure 1C represents the HPLC profile of the TDS1 mixture with Tat Bru.

Figure 1D represents the HPLC profile of the crude product of synthesis of Tat Oyi.

Figure 1E represents the HPLC profile of the mixture of the crude product of synthesis of Tat Oyi with 0.1 mM TDS1.

Figure 1E represents the HPLC profile of the mixture of the crude product of synthesis of Tat Oyi with 1 mM TDS1.

Figures 2A, 2B and 2C represent the mass spectra of three fractions collected after HPLC from the crude product of synthesis of Tat Oyi. Figure 2B corresponds to the mass spectrum of the major peak present in Figure 1D, but which has disappeared in Figures 1E and 1F.

Figure 3A represents the LTR-Lac Z activity of human cells infested with the LTR of HIV-1 and a *LacZ* reporter gene encoding beta-Galactosidase in the pressence of Tat Bru and TDS1.

Figure 3B shows the results of an experiment similar to that presented in Figure 3A where the reporter gene is replaced by *luc*, encodingluciferase.

Figure 4A represents the survival of MT4 cells in the presence of HIV-1 III B as a function of the concentration of TDS1, of the concentration of AZT and of the concentration of ddC.

Figure 4B represents the activity of reverse transcriptase at room temperature in the presence of HIV-1 III B as a function of the concentration of TDS1 and of the concentration of AZT.

Figure 5A represents the electrophoresis profiles of TAR, of the Tat Bru/TAR complex and of the Tat/TAR/TDS1 mixture after having incubated the Tat Bru protein with TDS1 for 30 minutes before the addition of TAR.

Figure 5B represents the electrophoresis profiles of TAR, of the Tat Bru/TAR complex and of the

Tat/TAR/TDS1 mixture after having incubated the Tat Bru protein with TAR before the addition of TDS1.

Figure 6A represents the electrophoresis profiles of TAR, of the Tat Mal/TAR complex and of the Tat/TAR/TDS1 mixture after having incubated the Tat Mal protein with TDS1 for 30 minutes before the addition of TAR.

Figure 6B represents the electrophoresis profiles of TAR, of the Tat Mal/TAR complex and of the Tat/TAR/TDS1 mixture after having incubated the Tat Mal protein with TAR before the addition of TDS1.

Figure 7 represents the fluorescence spectra of Tat Eli (curve 1), of the Tat Eli/TDS complex (curve 2) and of TDS1 (curve 3), after excitation at 295 nm.

Example 1: Preparation and activity of TDS1

A) PREPARATION

- **2,6,10-Trimethyltriphene**

2,6,10-Trimethyltriphene is obtained from 4-methylcyclohexanone according to the method of preparation described by SHIRAI et al. in J. Org. Chem., 56 (1991), 2253-2258.

The 4-methylcyclohexanone is reacted at 180-200°C in the presence of a catalytic quantity of $ZnCl_4$ in order to undergo autocondensation. The intermediate quadricyclic compound obtained is then dehydrogenated at 300°C on carbon/Palladium. The 2,6,10-trimethyltriphene is obtained with a yield of about 40%.

- **2-Bromo-4,6-dimethyltriphene:**

N-Bromosuccinimide (1.96 g; 11 mmol) and a catalytic quantity of AIBN are added to a solution of 2,4,6-trimethyltriphene (2.8 g; 10 mmol) in solution in dry CCl_4 (400 ml) degassed under nitrogen. The suspension is heated under reflux for 2 to 3 hours. The solvent is removed under vacuum and the residue is purified by chromatography on silica gel, eluting with a pentane/ethyl acetate gradient to give 1.7 g of monobrominated derivative (50%).

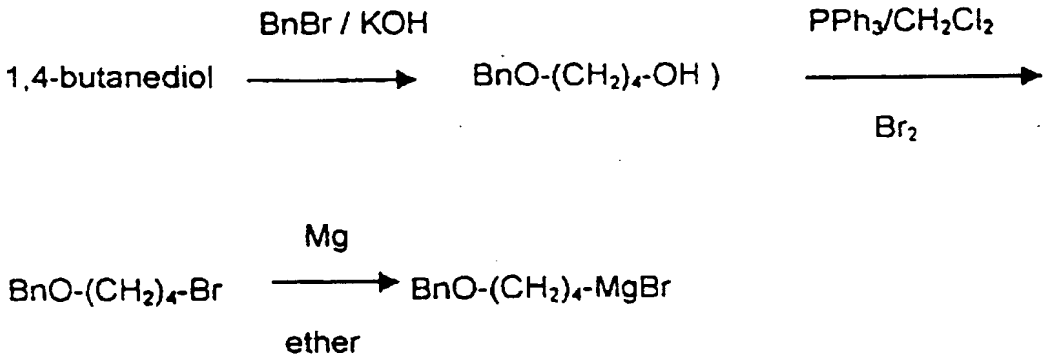
NMR (200MHz, $CDCl_3$): 2.58 (s, Me, 6H); 4.70 (s, CH_2Br, 2H); 7.5-8.5 (unresolved complex, aromat., 9H).

MS: 347 (M-H, 100); 268 (M-H-Bt, 95).

- **2-(5-Hydroxypentyl)-4,6-dimethyltriphene**

A catalytic quantity of a 1N solution of $CuLiBr_2$ (about 1 ml) is added to a solution of 2-bromo-4,6-dimethyltriphene (0.9 g; 2.6 mmol) in solution in dry THF under nitrogen at -78°C, followed by the slow addition (about 1 hour) of a 1.4N solution of $BnO-(CH_2)_4-MgBr$ (9 ml, 6.4 mmol).

$BnO-(CH_2)_4-MgBr$ is prepared according to the following scheme:



At the end of the addition, the reaction mixture is abandoned at room temperature overnight.

After conventional treatment of the reaction, the crude mixture is dissolved in a toluene/methanol mixture and a catalytic quantity of 10% Pd/C is added. This suspension is hydrogenated under a H_2 atmosphere for one day. The catalyst is filtered and the solution is concentrated. The crude mixture is purified by chromatography on silica gel, eluting with a pentane/ethyl acetate gradient to give 0.38 g of 2-(5-hydroxypentyl)-4,6-dimethyltriphene (yield of 43%).

NMR (200MHz, $CDCl_3$): 1.4-1.8 (unresolved complex, CH_2, 4H); 2.59 (s, Me, 6H); 2.87 (t, Ar-CH_2, 2H); 3.66 (t, O-CH_2, 2H); (unresolved complex, aromat. 9H).

- **TDS1:**

N-Succinimide (0.1 g; 1.0 mmol), diethylazocarboxylate (DEAD) (0.15 ml; 1.0 mmol) and triphenyl-

phosphine (PPh_3) (0.270 g; 1.0 mmol) are added to a solution of the preceding 2-(5-hydroxypentyl)-4,6-dimethyltriphene (0.28 g; 0.82 mmol) in solution in dry THF (20 mol) under nitrogen. The solution is stirred under nitrogen overnight at room temperature. After conventional treatment of the reaction, the solvent is removed and the crude mixture is purified by chromatography on silica gel, eluting with a pentane/ethylacetate gradient to give 0.2 g of TDS1 (50%).

NMR (200MHz, $CDCl_3$): 1.4-1.8 (unresolved complex, CH_2, 4H); 2.59 and 2.55 (s, Me and CH, -10H); 2.83 (, Ar-CH_2, 2H); 3.54 (t, N-CH_2, 2H); 7.5-8.5 (unresolved complex, aromat. 9H).

MS: 423 (M, 80); 269 (2,4,6-trimethyltriphene-H, 100).

B) ACTIVITY OF TDS1 in vitro

a) Test of interaction between Tat Bru and TDS1, and Tat Oyi and TDS1 carried out by HPLC.

Experimental conditions: C8 column, 20 to 60% gradient, buffer B (CH_3CN + 0.1%\TFA) over 40 min, buffer A (H_{20} + 0.1% TFA). The absorbance is measured at 215 nm.

Figures 1A, 1B and 1C represent respectively the HPLC profile of TDS1 (0.1 mM), Tat Bru (0.1 mM) and of the TDS1 mixture with Tat Bru.

These tests show that TDS1 causes a precipitation of Tat Bru in micromolar concentrations (Figures 1A, 1B and 1C).

An affinity which is at least similar is observed with the crude product of synthesis Tat Oyi (101 residues) (Figures 1D, 1E and 1F). The main peak of the crude product of synthesis of Tat Oyi possessing more or less substantial deletions of the N-terminal region which elutes at 16 min (Figure 1D) disappears completely when TDS1 is added at a concentration of 1 mM (Figure 1E) and of 10 mM (Figure 1F).

These results therefore show the specificity of TDS1 for Tat Oyi since the only main peak is modified by TDS1, whereas the derivatives having deletions or those having protective groups are not affected (Figure 1E and F).

The results presented Figure 1 show that TDS1 attaches directly to Tat Oyi. The experiments in the presence of the crude product of synthesis show that TDS1 attaches to Tat Oyi but not to the derivatives of Tat Oyi having a more or less large portion of the missing N-terminal end. Even in the presence of a high concentration of TDS1 (10 mM), the attachment of the molecule is still specific for whole Tat Oyi. Its specificity should make it possible to avoid undesirable secondary effects.

Furthermore, this experiment confirms that the N-terminal region of Tat is involved in the site of attachment.

b) Isolation Tat Oyi (MW 11561) by HPLC

Figure 2 shows the mass spectra of three fractions collected after HPLC from the crude product of synthesis of Tat Oyi. The first fraction corresponds to the small peaks observed before the major peak, and represents variants of Tat Oyi containing Tat mixed with variants possessing more or less substantial deletions of the N-terminal region (Figure 2A). The second fraction corresponds to the major peak, which is therefore identified as Tat Oyi since the observed MW of 11565 D corresponds to that expected for the whole protein (Figure 2B). The third fraction corresponds to the small peaks observed after the major peak, and represents derivatives of Tat Oyi with protective groups attached to side chains mixed with Tat Oyi (Figure 2c).

c) Inhibition of the transactivation with human HeLa cells transfected with the HIV-1 LTR and a reporter

gene for the beta-Galactosidase (Lac Z) or luciferase protein.

100 µl of TDS1 are added to a cell culture medium followed by 100 µl of the Tat Bru variant or of the Tat Mal variant.

The functional transactivation by the synthetic Tat protein is evaluated using HeLa-CD4 cells which carry a bacterial gene *lac Z* (or *luc*) under the control of the HIV LTR. The cytoplasmic accumulation of beta-Galactosidase (or of luciferase) depends on the presence of Tat. It has been possible to reproduce the inhibition of the transactivation in eight independent experiments with two different variants: Tat Bru (Figure 3A) and Tat Mal (Figure 3B). Similar results were obtained with the Tat Eli variant (result not shown).

These tests show that TDS1 inhibits the transactivation of HIV-1. The dose effect observed in Figure 3A indicates that TDS1 acts directly on Tat and not on a cellular cofactor which would be necessary for the transactivation.

The fact that TDS1 attaches equally well to Tat Bru, Tat Mal and Tat Eli confirms the presence of a specific site conserved in the variants of Tat.

The mean effect of inhibition of transactivation (IT_{50}) is 0.2 µM for Tat Mal (Figure 3B) for Tat Mal (Figure 3B). This value is of the same order for the other variants.

d) Survival of MT4 cells and reverse transcriptase activity (Figures 4A and 4B) in the presence of HIV-1 IIIB

During the two series of experiments, TDS1 shows that it can inhibit the cytotoxicity of HIV-1 on MT4 cells with a mean effect (IC_{50}) around 30 µM. AZT and ddC are respectively 1000 times and 100 times more active than TDS1 in this type of test. However, the fact that a reverse transcriptase inhibiting effect was observed with TDS1 was remarkable for a molecular which

attaches specifically to Tat. For example, AZT and ddC have strictly no effect in the transactivation test. The difference between IT_{50} on HeLa cells and IC_{50} on MT4 is also explained by the fact that the MT4 cells, modified by a virus other than HIV-1 so as to be able to be maintained in culture, are capable of expressing a Tat analogue called Tax. The difference in activity between the two cells types clearly shows the specificity of the inhibition for Tat. An important fact revealed by these experiments is that TDS1 is capable of crossing the membranes. The inhibitory effect of TDS1 cannot be explained otherwise.

e) Inhibition of the Tat-TAR interaction by TDS1

The RNA sequence of TAR of 59 nucleotides is prepared in vitro by transcription with T3 RNA polymerase. 20 µl of a mixture containing 0.2 nmol of radio labeled TAR, 0 to 100 ng of Tat and a buffer solution (50 mM Tris pH 7.4, 20 mM KCl, 0.1% Triton X-100) are also prepared.

The complexes are separated by electrophoresis on an 8% polyacrylamide gel containing 0.1% Triton X-100. The electrophoresis lasts for 90 minutes at 200 V. The relative quantities of free or bound RNA are determined by phosphor imaging.

The results are presented Figure 5 and Figure 6.

These experiments show that TDS1 is capable of inhibiting the Tat-TAR interaction although the affinity (Ka) of Tat for TAR (nM) is greater than the affinity of Tat for TDS1 (µM). This experiment shows the importance of allosteric inhibition because the Kd of TDS1 (100 pM) makes it possible to prevent the Tat/TAR interaction.

f) Interaction of TDS1 with tryptophan No. 11 of Tat Eli observed by fluorescence

The fluorescence spectrum presented in Figure 7 shows a transfer of energy between the triphene nucleus

of TDS1 and the aromatic nucleus of tryptophan No. 11 (Trp 11) of Tat Eli.

The principle of the method consists in exciting, at the same wavelength (295 nm), Tat Eli, the Tat Eli/TDS1 complex and TDS1. The fluorescence of these three compounds is observed from 340 to 450 nm.

The observation of the three curves Tat Eli (curve 1), Tat Eli/TDS1 (curve 2), TDS1 (curve 3) shows that the fluorescence of the Tat Eli/TDS1 complex does not correspond to the superposition of the curves of Tat Eli and TDS1. In particular, the strong band observed at 365 nm indicates an exciton effect between the aromatic nucleus of Trp 11 of Tat Eli, and that of the triphene of TDS1. This exciton effect can be explained only by the parallel positioning of the two nuclei at an approximate distance of 0.2 +/- 0.05 nm.

Successive dilutions of the Tat Eli/TDS1 complex show that the complex is still stable at 1 nanomol/liter and that the molecules are completely separated at a concentration of 10 picomol/liter. The dissociation constancy (Kd) of the complex is estimated at around 100 picomol/liter.

These experiments show that when the Tat Eli/TDS1 complex is formed, the reaction is almost irreversible, which explains why TDS1 is capable of preventing the formation of the Tat Eli/TAR complex (Kd = 50 nanomol/liter).

In addition, modeling of the interaction between Tat Bru and TDS1, using a 2D NMR structure preserving 950 NMR constraints, indicates that the triphene nucleus of TDS1 probably positioned itself between the aromatic nucleus of Trp 11 and that of Phe 38 of Tat Eli. This interaction is very strong and similar to that observed between the nucleotide basis (phenomenon of "stacking" of the pyrimidine and purine nuclea in the nucleic acids). The positioning in "sandwich" form of the triphene nucleus makes it possible to assume that the two proton donors

interacting with the maleiminde nucleus, are the NH of the peptide bond of Arg 7 and Arg 52.

g) Toxicity study in rats

mM doses of TDS1 were injected subcutaneously into young rats. No toxicity was observed during the injection and the animals have had a normal growth for six months. The choice of young rats during the injection makes it possible to more easily detect mutagenic effects. No tumor has been observed up until now. In coculture with human cell lines, toxicity starts to be detected from 100 µM TDS1. This toxicity could be linked to the hydrophobic solvent.

Example 2: Preparation of TDS2

· 2,6-10-Tri(hydroxymethyl)triphene (2)

2,6,10-Trimethyltriphene (1) is prepared as in Example 1.

According to a first variant, 2,6,10-trimethyltriphene (1) is added to 3.3 equivalents of N-bromosuccinimide (NBS) in CCl_4, under reflux for 2 to 3 hours, in the presence of a catalytic quantity of AIBN. The tribrominated derivative is then hydrolyzed in a basic medium by the action of KOH, in an $MeOH/H_2O$ mixture, at room temperature for 24 hours. 2,6,10-Tri(hydroxymethyl)triphene is obtained with a yield of 50%.

According to a second variant, 2,6,10-trimethyltriphene (1) is exposed to 3.3 equivalents of $Na_2Cr_2O_7$ in aqueous medium, at 250°C, under pressure, for 13 hours. The triacid obtained is then reduced with $AlLiH_4$, in THF at 0°C, for 2 hours. The yield is 80%.

· 2-Bromomethyl-6,10-di(tert-butyldimethylsilyloxy)-triphene (2bis)

2,6,10-Tri(hydroxymethyl)triphene (318 mg; 1 mmol) is reacted with 2 equivalents of tert-butyldimethylsilyl chloride (TBSCl) (0.3 g; 0.2 mmol)

in CH_2Cl_2 in the presence of imidazole (0.17 g; 2.5 mmol).

The derivative obtained is brominated by the action of Br_2 in CH_2Cl_2 in the presence of PPh_3 (yield 50%)

· 2-(5-Benzyloxypentyl)-6,10-di(hydroxymethyl)triphene (3)

The brominated derivative obtained above and $CuLiBr_2$ are dissolved in THF. The mixture is cooled to -78°C and then reacted with $BnO-(CH_2)_4-MgBn$.

The tert-butyldimethylsilyl protective groups are hydrolyzed with nBu_4NF in THF.

· TDS2

The dihydroxylated derivative (3) is treated with Br_2 in CH_2Cl_2 in the presence of PPh_3. The corresponding dibrominated derivative (4) obtained is then reacted with the aminodiol (5) in DMF in the presence of Et_3N.

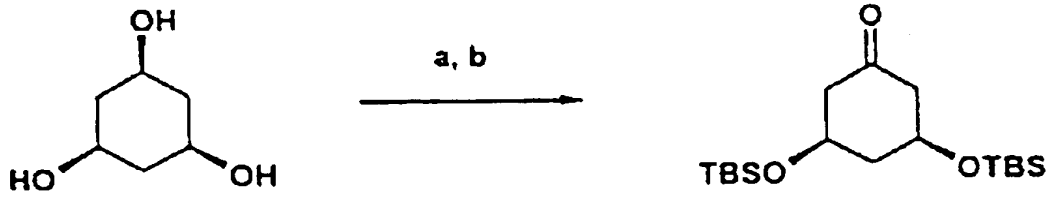
The hydrogenolysis of the benzyl group and the hydrogenation of the two double bonds are carried out during the same step. The deprotected alcohols direct the hydrogenation of the double bond such that the desired stereochemistry is obtained.

The derivative (7) may also be obtained according to another method: by oxidizing the two alcohol functions of the derivative (3) of Example 2 to an aldehyde and then by reacting the aldehyde with 3,5-di(OTBS)cyclohexanone.

Example 3: Preparation of TDS3

The dibrominated derivative (4) obtained according to Example 2 is exposed to PPh_3 in DMF; nBuLi is added in order to form a ylide, followed by the ketone (6).

The ketone (6) is prepared according to the following scheme, from the hexane-trial:



a) 2 equivalents TBSCl, imidazole, CH_2Cl_2 b) Swern oxidation.

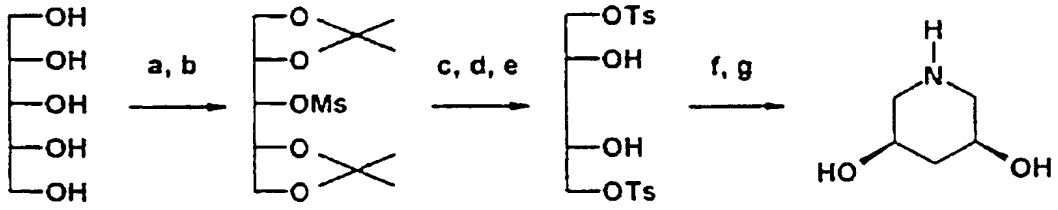
The derivative obtained by the preceding Wittig reaction (7) is then treated with NBu_4NF in THF in order to deprotect the two alcohol functions. The hydrogenolysis of the benzyl group and then the action of NBS are carried out under the same conditions as in Example 2.

Example 4: Preparation of TDS4

The 2-bromomethyl-6,10-di(tert-butyldimethylsilyloxy)triphene derivative (2bis) is prepared as in Example 2 and then dissolved in THF with $CuLiBr_2$. The mixture is cooled to -78°C and then reacted with BnO-$(CH_2)_4$-MgBr. The benzyl group is hydrogenolyzed under the same conditions as those of Example 2 and then the succinimido group is grafted onto the pentyl chain under the same conditions as those of Example 1.

In a last step, the tert-butyldimethylsilyl protective groups are hydrolyzed with nBu_4NF in THF.

The aminodiol (5) is obtained from the adonitol HO-CH_2-$(CHOH)_3$-CH_2OH according to the following scheme:



a) acetone, TsOH catalysis b) methanesulfonyl chloride (MsCl), Et_3N, CH_2Cl_2, c) $LiAlH_4$, THF d) TsOH catalysis, MeOH e) p-toluenesulfonyl chloride (TsCl), pyridine, CH_2Cl_2 f) benzamide ($BnNH_2$), dioxane, reflux g) 1 Atm, H_2, Pd/C 10%, MeOH.

The benzyl group is hydrogenolyzed in order to release the primary alcohol function, by the action of the dihydrogen (1 atm.) on 10% Pd/C catalysts, in an MeOH/toluene mixture.

Finally, the succinimido group is grafted onto the pentyl chain by the action of NBS under the same conditions as in Example 1.

Example 5: Two routes of preparation of aromatic derivatives substituted with a 5-succinimidopentyl chain

a) An aromatic derivative ArH such as toluene, anthracene or triphene is reacted with glutaric anhydride, in CH_2Cl_2, at 0°C, in the presence of $AlCl_3$. The toluene is substituted at the 4-position, the anthracene at the 9-position, and the triphene at the 2-position. The benzylic carbon is reduced in order to obtain Ar-$(CH_2)_4$-COOH by the action of $Zn(HgCl_2)$, for 48 hours under reflux in a toluene/water/concentrated HCl mixture.

The carboxylic acid is reduced to an alcohol with $AlLiH_4$ in ether at 0°C for 2 hours and then the succinimido group is grafted under the conditions of Example 1.

b) The triphene is brominated at the 2-position according to the Barker et al. method (J. Chem. Soc., 1955, 4482-4485). The brominated derivative is then reacted with ZnI-$(CH_2)_4$-CN in the presence of nickel (0) as catalyst. The nitrile obtained is hydrogenated with H_2 on Pd/C in ethanol and then exposed to succinic anhydride.